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20 Dec. 01

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13. ABSTRACT ***(Maximum 200 Words)***

This study was carried out to test the hypothesis that RAS activation could contribute to prostate tumor radiation resistance and that farnesyltransferase inhibitor treatment of prostate tumor cells expressing activated *ras* oncogenes would reduce their clonogenic survival after irradiation. The studies undertaken have shown increased radiation survival in many, but not all prostate tumor cell lines expressing activated RAS proteins. Treatment of these cell lines and tumors derived from a subset of these cell lines with farnesyltransferase inhibitors resulted in decreased radiation survival both in *in vitro* assays and when these cells were used to generate tumors in athymic nude mice. It was further shown that tumor oxygenation was enhanced after farnesyltransferase inhibitor treatment. Radiosensitization by farnesyltransferase inhibitors appears limited to those cells with activated *ras*. Further study will be needed to determine whether farnesyltransferase inhibitors can affect the radiosensitivity of prostate tumors expressing wild-type *ras in vivo*. Taken together these results demonstrate the potential of farnesyltransferase inhibitors in the treatment of certain prostate tumors.

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Table of Contents

Introduction:

Oncogenic *ras* expression and over-expression of wt *ras* has been shown to increase the radiation resistance of many human and rodent tumor cell lines(1-4). The focus of this grant is to examine whether the expression of activated *ras* in prostate tumors increases their resistance to killing by radiation, and if this is the case, to examine a means of reversing this radiation resistance. This grant was funded without modification of the specific aims which are:

Aim 1. To determine the effects of prenyltransferase inhibition on the radiosensitivity of H-ras transformed murine prostate tumor cells.
Aim 2. To define the effects of prenyltransferase inhibition on the radiosensitivity of human prostate tumor cell lines expressing either H- or K-*ras* oncogenes.
Aim 3. To determine the effectiveness of prenyltransferase inhibitors as prostate tumor radiosensitizing agents *in vivo*.

We have previously shown that transformation by oncogenic *ras* caused increased radiation resistance in rat embryo fibroblasts (5). We subsequently showed that inhibition of RAS activity led to a decrease in the radiation survival in *ras*-transformed cells accompanied by an increased radiation-induced apoptosis (6). In these studies, RAS activity was inhibited by preventing the post-translational prenylation of the RAS protein that is required for its membrane association. Subsequent studies on human tumor cells with endogenous H- or K-*ras* mutations showed that these cells could also be radiosensitized by prenyltransferase inhibitors *in vitro* (7). Farnesyltransferase inhibitors were also shown to be effective radiosensitizing agents *in vivo* (8), and more recently shown to cause enhanced oxygenation of otherwise hypoxic tumors expressing activated RAS (9). Activating mutations of *ras* genes have been found in both human prostate tumors (10-15)] and have been studied in animal models of prostate tumorigenesis (16-18). We have therefore examined the role of RAS activation in prostate tumor cell radioresistance.

Body:

RAS effects on radiation survival *in vitro*.

As outlined in the plan of work, the majority of the work during the first funding year focussed on specific aims 1 and 2. During the second period of funding, we have expanded our observations *in vitro*. We have also addressed the efficacy of FTI treatment on the radiosensitivity of prostate tumor grafts *in vivo* (Specific aim 3). As a result of observations made in mouse prostate tumors and other tumor systems (9), and in addition to the work proposed in the plan, we have also examined the effects of FTI treatment on the prostate tumor micro-environment.

In order to characterize the influence of activated RAS on the radiation resistance of prostate tumor cells we have inhibited H-RAS activity in mouse prostate tumor lines expressing this protein using farnesyltransferase inhibitors. These inhibitors block the post-translational processing of the ras p21 protein required for its association with the cell membrane and its activity. The inhibition of RAS processing is measured by examining RAS migration on an SDS-polyacrylamide gel using western blotting with RAS specific antibodies to demonstrate the change in migration (Figure 1).

Our studies have confirmed the preliminary results presented in the original application showing that treatment of H-*ras* + v-*myc* transformed mouse prostate tumor cells with farnesyltransferase inhibitors can result in increased apoptosis and significantly decreased long-term survival after irradiation as

measured by clonogenic assay. These results have now been obtained with two different inhibitors from two independent sources. A total of 4 murine prostate tumor cell lines have been examined for induction of apoptosis by FTI plus radiation. Of these 4, three show significant enhancement of apoptosis at 24 h after irradiation although the levels of apoptosis varied between cell lines. Representative data are shown for two of the cell lines in Figure 2. One cell line showed no significant difference from irradiation alone after farnesyltransferase inhibitor treatment (not shown).

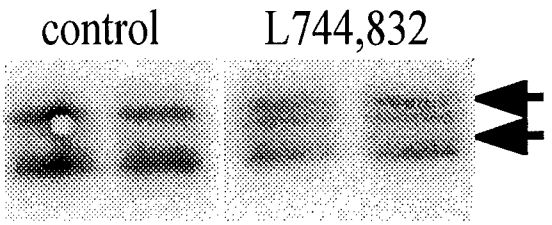

Figure 1. Inhibition of H-RAS farnesylation by FTI treatment *in vitro*. 141-1primary(B) mouse prostate tumor cells were treated with 5μM L744,832 for 24h. Cells were then lysed and proteins analysed by Western blotting using an H-RAS specific antibody. Two species of RAS are present in untreated cells. Both the fast and slow migrating species of RAS are shifted after FTI treatment.

The clonogenic survival of mouse prostate tumor cell lines has also been examined after treatment with the L744,832 inhibitor (Figure 3 and Table 1). The radiation survival of mouse prostate tumor cells as assessed by limiting dilution analysis was decreased after combined L744,832 inhibitor and 2 Gy irradiation treatment to a greater extent than was seen with either treatment alone. The radiation survival of tumor cell clonogens shown in Figure 3 was reduced by 15-20% when corrected for the toxicity of the drug alone.

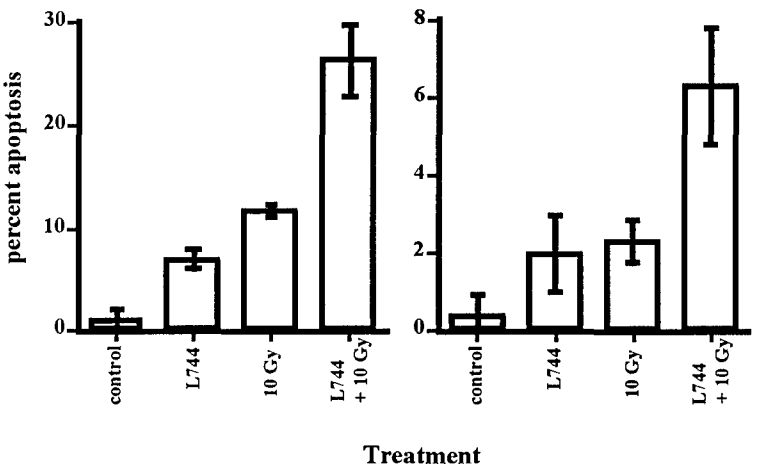

Figure 2. FTI treatment augments radiation induced apoptosis. Murine prostate lines 148#1primary tumor(B) (left panel) and 151#2 lung metastasis(D) (right panel) were treated for 24 h prior to irradiation with 2μM L744,832. Apoptosis was determined by examining nuclear morphology after staining with propidium iodide. Although both lines show significant increase in apoptosis after irradiation that is further increased by farnesyltransferase inhibitor treatment, the absolute levels of apoptosis vary between cell lines.

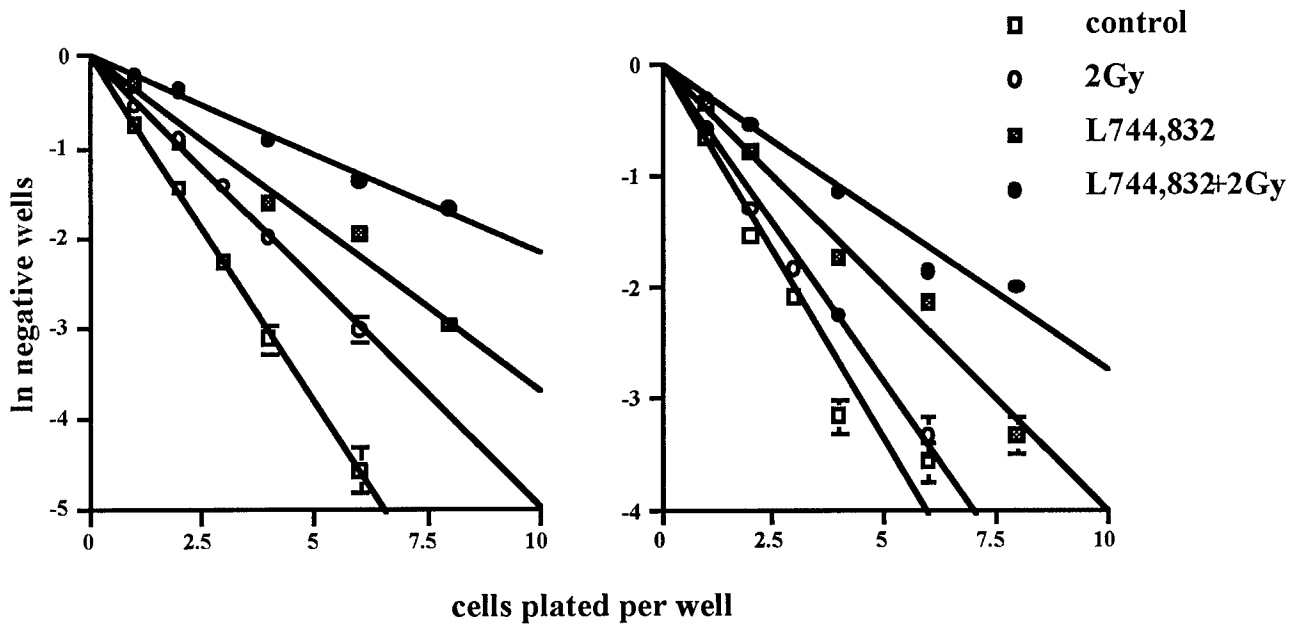

Figure 3. Clonogenic survival of two prostate tumor lines determined by limiting dilution analysis after treatment with the farnesyltransferase inhibitor L744,832. In this analysis the steeper the slope of the line, the greater the plating efficiency of the cells. Clonogenic survival is determined in replicate 96 well plates at a single radiation dose of 2 Gy. The surviving fraction is determined by dividing the slope of the irradiated cells by the slope of the appropriate controls. The surviving fraction at 2 Gy of 151#2 primary (C) (Left panel) was decreased from 0.61 to 0.52 by FTI treatment. The surviving fraction at 2 Gy of 151#2 lung metastasis (D) (right panel) was decreased from 0.85 to 0.68 in this experiment.

Although magnitude of the effect of farnesyltransferase inhibitor treatment on apoptosis and radiation survival varies between mouse cell lines, the data we have obtained confirm that farnesyltransferase inhibitors can increase radiation-induced apoptosis and decrease clonogenic survival in this model of prostate cancer.

We have extended our studies to human cells expressing oncogenic H- or K-ras as a result of transfection. We successfully obtained clones of the 267B1 cell line (19) after transfection with H-ras that expressed high levels of H-ras^{V12}. These clones showed alterations in morphology including greater refractility and rounding up of the cells and initially showed a two-fold increase in radiation survival from 0.4 to 0.7-0.8 after H-*ras* transfection (Figure 4 and Table 2). FTI treatment of these cells resulted in altered H-RAS migration (Figure 5) and resulted in the reduction of this radiation resistance to that of the parent cells (Figure 6 and Table 2). Thus introduction of oncogenic H-*ras* into human prostate cells can increase radiation resistance, and this resistance can be reversed by inhibiting RAS farnesylation.

Table 1. Surviving fraction of murine prostate tumor clonogens after 2 Gy irradiation and treatment with farnesyltransferase inhibitors		
Cell line:	SF_2 control	SF_2 with FTI (inhibitor used)
148#1 primary (B)	1.08*	0.68* (5μM FTI-277)
148#1 lung metastasis (A)	0.89*	0.54* (5μM FTI-277)
151#2 primary (C)	0.61 0.63 0.73*	0.52 (2μM L744,832) 0.25 (5μM FTI-277) 0.59* (5μM FTI-277)
151#2 lung metastasis (D)	0.85 0.90	0.68 (2μM L744,832) 0.38 (5μM FTI-277)
Data were obtained by limiting dilution analysis as in Figure 3 excepting values with an asterix. Those data are extracted from survival curves at the 2 Gy dose point where survival is defined as: Number of colonies formed / (Number of cells plated)*(Plating efficiency) Both methods yield equivalent values.		

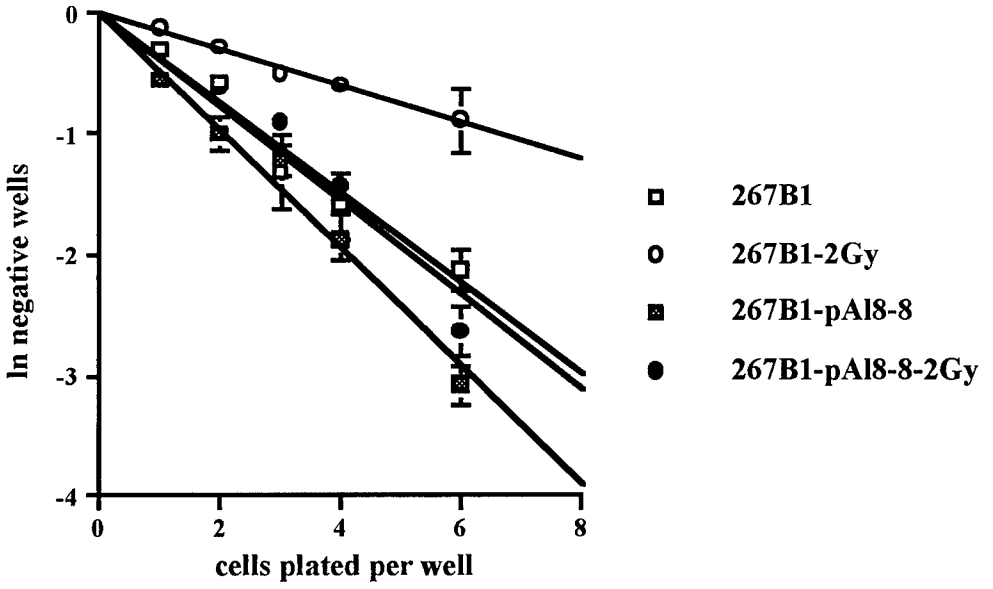

Figure 4. H-ras transfection increases clonogenic survival after irradiation. The radiation survival of parental 267B1 cells was compared to that of 267B1 cells transfected with the pAL8 plasmid encoding activated H-ras. Radiation survival, as determined by limiting dilution analysis as in Figure 2 was increased from a surviving fraction of 0.4 to 0.8 after transfection with a plasmid encoding H-ras^{V12}.

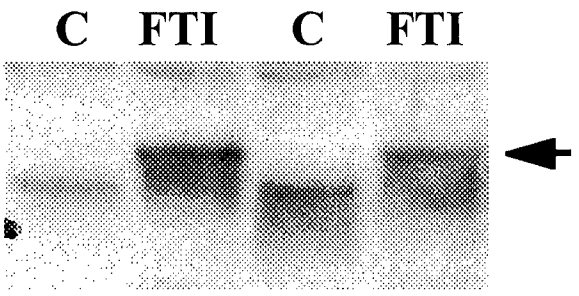

Figure 5. Altered migration of H-ras protein after FTI treatment of human prostate cells. The 267B1pAL8 clone expressing high levels of H-ras^{V12} before (C) and after (FTI) farnesyltransferase inhibitor treatment with L744,832 at 2.5μM for 24 h. Unfarnesylated H-ras (arrow) migrates more slowly.

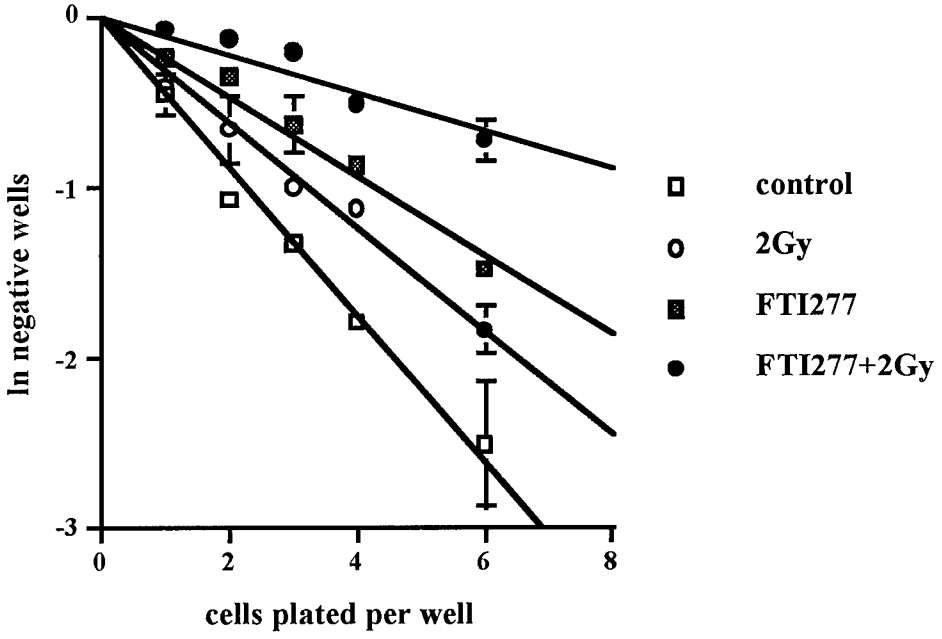

Figure 6. FTI treatment reduces clonogenic survival in human prostate cells expressing activated H-RAS. 267B1-pAL-8 cells expressing high levels of H-ras^{V12} were tested for clonogenic survival after treatment with FTI-277. Radiation survival as assessed by limiting dilution analysis in this experiment was reduced from a surviving fraction of 0.7 to 0.48 after FTI-277 treatment.

Sensitization of the TSU-Pr-1 cell line expressing an endogenous activated H-*ras* allele was also seen also this cell line displayed a relatively radiosensitive phenotype. In contrast, radiosensitization of another relatively radiosensitive cell line, the Ki267B1 cell line expressing activated K-ras, was not seen after FTI treatment (not shown). As predicted from our hypothesis that RAS activation contributes to radioresistance, radiosensitization of prostate tumor cell lines expressing wild-type ras was also not observed (Table 2).

Phosphotidyl-inositide-3-kinase (PI3K) has been implicated in RAS-mediated radiation resistance (20, 21). We therefore examined whether inhibition of PI3K would radiosensitize prostate tumors expressing activated RAS. As shown in Table 2, the PI3K inhibitor LY294002 reduced radiation survival in TSU-Pr1 cells to a similar extent as seen after FTI treatment. In contrast radiosensitization of prostate tumor cells expressing wt- *ras* was not observed.

Table 2. Surviving fraction of human prostate tumor clonogens after 2 Gy irradiation and treatment with farnesyltransferase or PI3kinase inhibitors

Cell line:	SF2 control	SF2 with FTI (inhibitor used)
267B1	0.56 0.45	0.56 (2μM L744,832) 0.55 (5μM L744,832)
267B1pAL8-8	0.7 0.7	0.62 (5μM L744,832) 0.48 (5μM FTI-277)
TSU-Pr1	0.48	0.35 (5μM L744,832) 0.36 (10μM LY294002)
PC3	0.63*	0.50* (5μM L744,832)
DU145	0.74 0.62*	0.89 (5μM L744,832) 0.65* (10μM LY294002)
LnCAP	0.24	0.18 (5μM L744,832) 0.24 (10μM LY294002)
Data from limiting dilution analysis and *data extracted from survival curves at the 2 Gy dose point as in Table 1.		

RAS effects on radiation survival and tumor oxygenation *in vivo*.

Having established an effect for FTI treatment on the radiosensitivity of prostate tumor cells expressing activated RAS *in vitro* we next examined the effect of FTI treatment on the radiosensitivity of tumor xenografts derived from these cells. Two parameters were examined: clonogenic survival of tumor cells isolated from tumors treated with FTI and irradiated *in vivo*, and the regrowth of tumors after sub-curative treatment with FTI and radiation. The ability of FTI treatment to inhibit RAS farnesylation *in vivo* in the tumors used for these studies was first established. Inhibition of H-RAS farnesylation was seen in both the murine and human prostate tumor grafts after 3 days FTI treatment as shown in Figure 7.

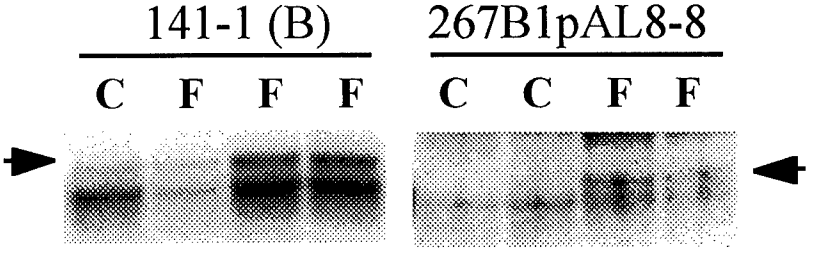

Figure 7. Inhibition of H-RAS farnesylation *in vivo*. Tumor-bearing mice were treated for 3 days with 40mg/kg/d L744,832 (F), or with vehicle (C) using ALZET mico-osmotic pumps for 3 days prior to sacrifice as described previously (8). Protein lysates obtained from tumors were analysed for RAS expression and migration by Western blotting as described in Figure 1.

Clonogenic survival of prostate tumor cells after irradiation *in vivo* was then examined. Mice were treated with L744,832 for 3 days as described in Figure 7 after which tumors were irradiated, immediately excised and dissociated into a single cell suspension and plated in culture dishes for colony formation (8). Irradiation with 8 Gy reduced clonogenicity 2-fold from 0.72 to 0.36. Irradiation after FTI treatment reduced clonogenicity 6-fold from 0.48 to 0.008. Thus, the effect of FTI treatment reduced clonogenicity after irradiation in a super-additive manner. The total reduction in clonogenicity with combined FTI and radiation exposure relative to controls was 9-fold.

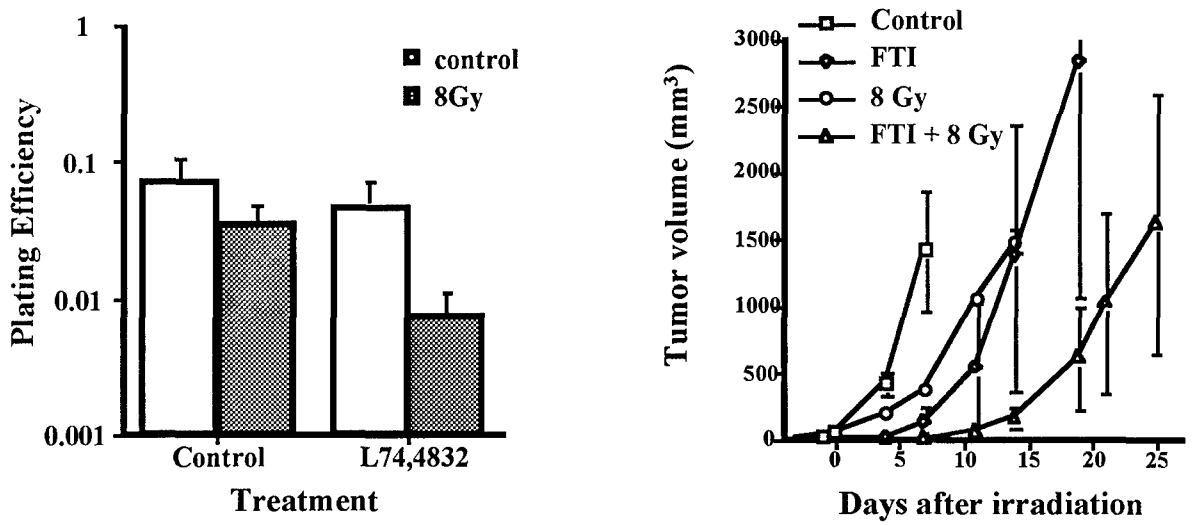

Figure 8. Radiosensitization of murine prostate tumors. Clonogenic survival of tumor cells (left) and tumor regrowth delay (right) were measured in 141-1(B) tumor bearing animals after continuous infusion treatment with 40mg/kg/d L744,832 for 3 days (left) or 20mg/kg/d L778,123 for 7 days (right). Clonogenic survival data are pooled results from 2-4 individual tumors of 300-400mm^3 at the time of the experiment. Tumor regrowth was evaluated in mice with established tumors of 22+/-9mm^3 volume. Each group contained a minimum of 6 mice.

The reduction in tumor cell clonogens after FTI treatment was also reflected in a delay of tumor regrowth after irradiation. Control tumors attained 1500mm^3 by d 7 after the start of this experiment. Tumors treated with L778,123 or radiation showed a delay of 7 days to attain this size. Tumors treated with a combination of both FTI and radiation showed a further delay of 18 days attaining a size of 1500mm^3 by d 25. Thus the delay obtained with combined treatment was greater than the sum of the delays obtained with either treatment alone.

Similar results were obtained using the human prostate cell line 267B1pAL8-8 (Figure 9). While FTI treatment alone had little effect on plating efficiency, Plating efficiency after FTI treatment and 6 Gy was reduced over 10-fold (0.00019) relative to the reduction obtained with irradiation alone (0.0021). Regrowth of these tumors was also slowed by FTI treatment. FTI treatment alone caused only a 4 day delay in tumor growth to 300mm^3. Irradiation to 6 or 8 Gy delayed tumor growth by 22 to 23 days respectively. Irradiation of FTI treated tumors delayed the time to attain 300mm^3 relative to control tumors by 29 days for 6 Gy irradiated mice and 35 days for 8 Gy irradiated mice. At both 6 and 8 Gy the effects of FTI + radiation were more than the sum of either treatment alone.

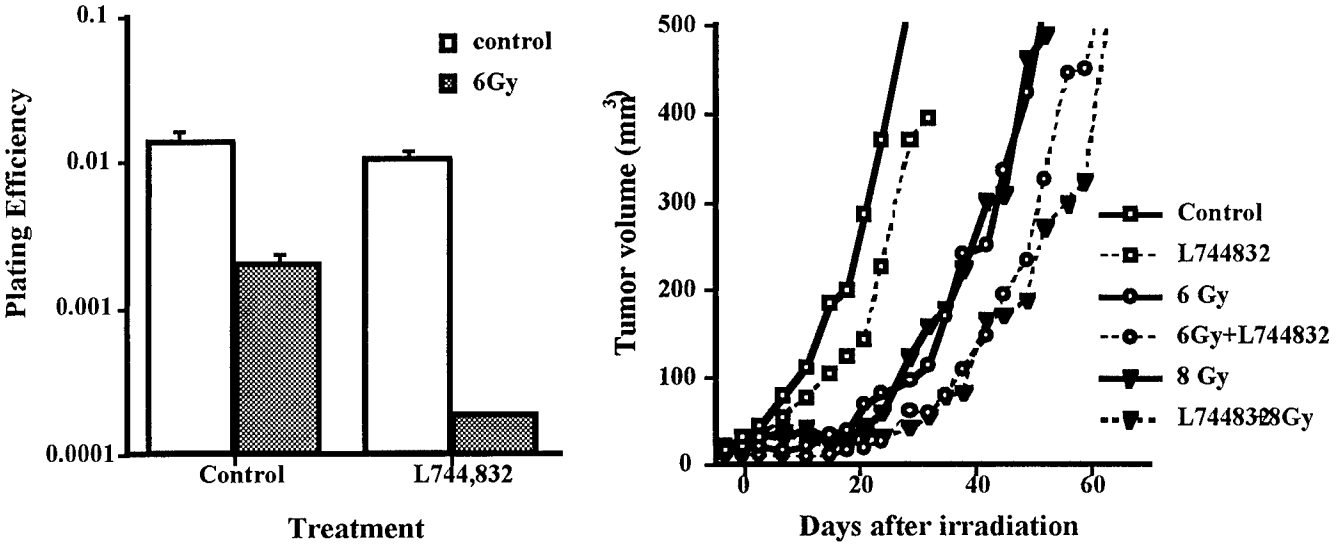

Figure 9. **Radiosensitization of human prostate tumor xenografts.** Clonogenic survival of tumor cells (left) and tumor regrowth delay (right) were measured in 267B1pAL8-8 tumor bearing animals after continuous infusion treatment with 40mg/kg/d L744,832 for 3 days (left) or for 7 days (right). Clonogenic survival data are pooled results from 3-4 individual tumors of 300-400mm^3 at the time of the experiment. Tumor regrowth was evaluated in mice with established tumors of 14-23 mm^3 volume. Each group contained 4-6 mice except the 8 Gy + FTI group that contained 3 mice.

We have also examined the effects of treating prostate tumor xenografts with FTI on the tumor microenvironment. Mouse prostate tumors growing sub-cutaneously were labeled with the hypoxic cell marker EF-5 (22, 23). Binding of this marker was detected with a cy-5 conjugated monoclonal antibody specific for EF-5 (22). Vessels were subsequently stained with the endothelial cell marker anti CD-31 monoclonal antibody. Areas of hypoxia are seen as red staining cellular regions in these sections. Vessel distribution is detected as fillamentous green staining. As shown in Figures 10 and 11, prostate tumor grafts are hypoxic. Treatment for 7 days with the L744,832 farnesyltransferase inhibitor resulted in markedly higher oxygenation of both 141-1(B) mouse (Figure 10), and 267B1pAL8 human (Figure 11) prostate tumors. This is important in that tumor cell oxygenation can have a large influence on the response of these cells to radiation (reviewed in (24)). A severely hypoxic environment can result in a two-fold or greater reduction in tumor cell killing by radiation. Thus farnesyltransferase inhibitor treatment of these tumors resulted in a microenvironment that is predicted to result in improved tumor cell killing by radiation.

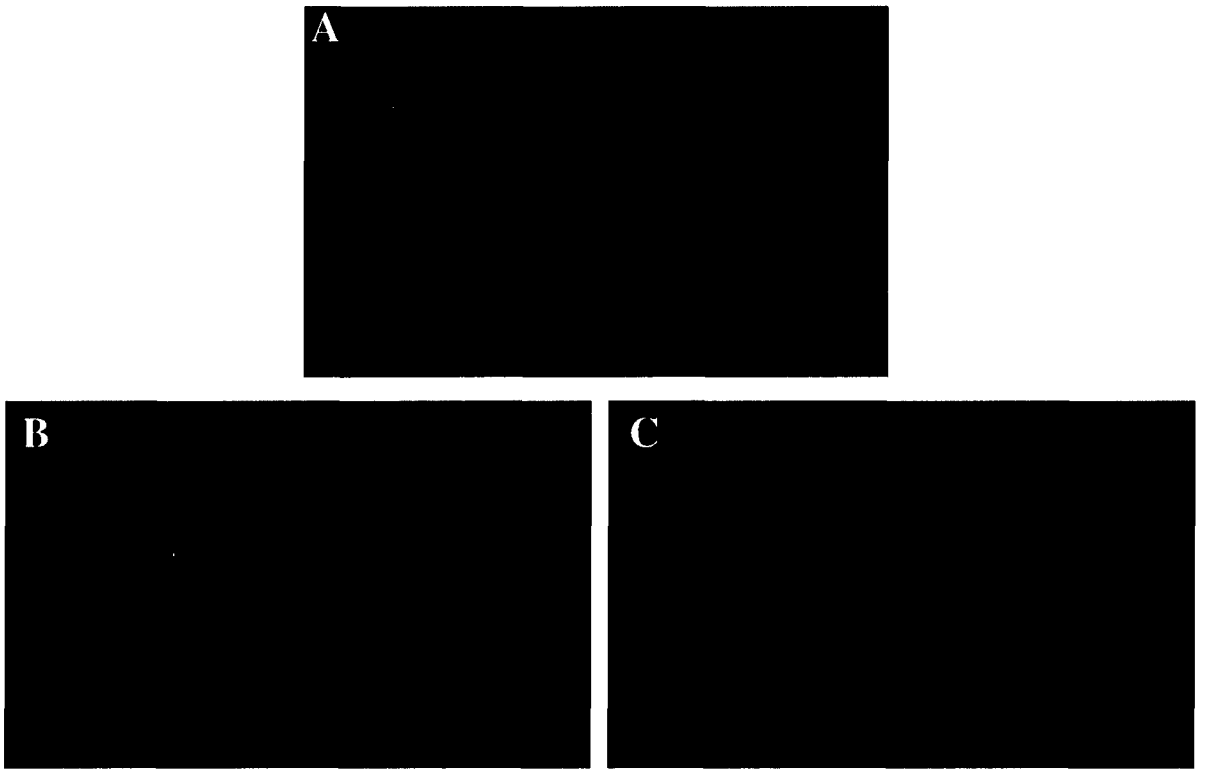

Figure 10. Enhanced oxygenation after FTI treatment of 141-1(B) tumor grafts. Tumor bearing animals were treated for 7 days by continuous infusion s.c. with carrier (A), with 30mg/kg/d L778,123 (B) or with 40mg/kg/d L744,832 (C). Exposure times for EF-5 fluorescence detection (red) were: 0.74sec (A), 0.8sec (B) and 3.71sec (C). The exposure for (C) was therefore 5x that for control cells. Sections are counter-stained with anti-CD31(green)

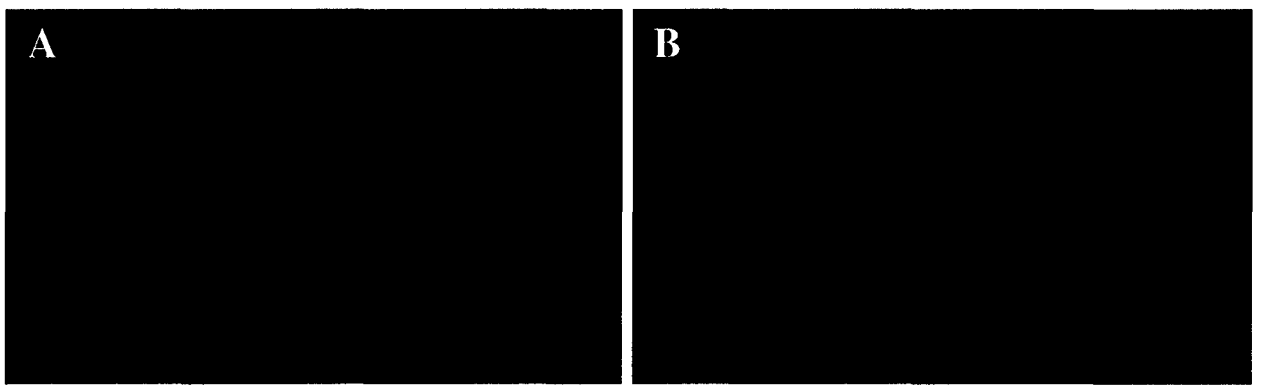

Figure 11. Enhanced oxygenation after FTI treatment of 267B1 tumor xenografts. Tumors were treated as above for 6 days with carrier (A) or with 40mg/kg/d L744,832 (B). Exposure times for EF-5 fluorescence detection were: (A), 0.52sec (B) and 4.0sec. Sections are counter-stained with anti-CD31(green)

Key Research Accomplishments:

1. We have shown that the expression of activated RAS can increase radiation resistance in both a rodent and a human cell system, although an increase in radiation resistance in prostate cells expressing oncogenic *ras* was not a universal finding.
2. When increased radiation resistance was found after oncogenic *ras* introduction, this resistance was abrogated through inhibition of RAS activity using farnesyltransferase inhibition.
3. Three inhibitors have now been tested on prostate cells, the FTI-276 inhibitor developed by Dr. Andrew Hamilton (now at Yale), and the L744,832 and L778,123 inhibitors developed by Merck and Co. Inc. With the exception of one experiment on DU145 cells expressing wt *ras*, the inhibitors displayed similar radiosensitizing effects.
4. We have demonstrated *in vivo* radiosensitization of H-ras^{V12} expressing murine and human prostate tumor xenografts.
5. We have shown enhanced oxygenation of prostate tumors after treatment with farnesyltransferase inhibitors. This is a novel finding and predicts a positive effect for FTI treatment on the response to radiotherapy and could possibly affect metastasis and response to chemotherapy as well.

Reportable Outcomes:

Development of new cell lines derived from immortalized human prostate epithelial cells that express high levels of H-ras^{V12}.

Publication of data relating to prostate tumor reoxygenation in Cancer Research 61:2289-93 (2001) and inclusion of this data in monograph chapters.

Publication of abstracts and oral presentation of results of studies on prostate cell lines and tumor xenografts.

Preparation of a manuscript to cover the work accomplished under this grant.

Training of medical student supported by an NIH short-term research grant for medical student training obtained on the basis of her work on this project. (Kun Huang).

Training of a post-doctoral fellow (Yuquan Shi, MD, PhD.) for the last 7 months of the grant period.

List of personnel supported by grant

Eric Bernhard, Ph.D. principal investigator

JunMin Wu, B.Sc. research specialist

List of personnel receiving training who's work was in part supported by this grant

Kun Huang, MD

Yuquan Shi, MD, Ph.D.

Publications, meeting abstracts and invited lectures:

Publications:

Cohen-Jonathan, E., S.M. Evans, C.J. Koch, R.J. Muschel, W.G. McKenna, J.M. Wu and E. J. Bernhard. 2001. The Farnesyltransferase Inhibitor L744,832 Reduces Hypoxia in Tumors Expressing Activated H-ras. Cancer Res. 61: 2289-2293

Bernhard, E.J., Muschel, R.J., Cohen-Jonathan, E. , Favre, G., Hamilton, A.D., Sebti, S., and McKenna, W. G.2000. Prenyltransferase inhibitors as radiosensitizers. In *Prenyltransferase Inhibitors in Cancer and Cardiovascular Therapy.* S.M. Sebti and A.D. Hamilton, eds. Humana Press Inc. Totowa, NJ.

Meeting abstracts:

Cohen-Jonathan, E., Evans, S.M., Koch, C., .Muschel, R.J, McKenna, W.G. Gibbs, J.B. and Bernhard, E.J. The farnesyltransferase inhibitor l744,832 induces reoxygenation in tumors expressing activated H-ras. 91st AACR Annual Meeting San Francisco, CA. April 1-5, 2000.

Bernhard,E.J., Cohen-Jonathan, E., Evans, S.M., Koch, C., Gibbs J.B., Muschel, R.J. and McKenna,W.G. The reoxygenation of tumor xenografts expressing activated H-ras after farnesyl-transferase inhibitor treatment. 47th Annual meeting Radiation Research Society, Albuquerque, NM. April 29th-May 3rd 2000.

Lectures by Invitation:

Symposium presentation: Farnesyltransferase inhibitors and the elimination of hypoxic tumor cells. 92nd Annual Meeting AACR, New Orleans LA. March 27, 2001

The effect of prenyltransferase inhibitors on radiosensitivity, cell signaling and tumor response. 16th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. , Les Menuires, France. March 5, 2001

Farnesyltransferases and Radiosensitization, Paul Scherrer Institute, Villigen, Switzerland June 27, 2000

The effects of oncogenic ras on intrinsic radiosensitivity

and the tumor microenvironment. International 4th Wolfsburg meeting on Molecular

Radiation Biology/Oncology. Ermatingen, Switzerland. June 24-26, 2000

Farnesyltransferase inhibitors: is the target we aimed for the target we want? Results of preclinical and

phase I studies. Oncology Day Symposium, Netherlands Cancer Institute

Amsterdam, The Netherlands. June 20, 2000

Conclusions:

We have, during the funding period of this grant, demonstrated that the expression of activated H-RAS can lead to increased radiation resistance in both rodent and human prostate tumor cells. The radiation resistance induced by H-*ras* activation was reversed by treatment with farnesyltransferase inhibitors. Reduced radiation resistance was also demonstrated *in vivo*. These data support the hypothesis of the grant that RAS activity can contribute to radiation resistance. It should be noted however, that radiation resistance was not a universal finding in prostate cells expressing activated RAS. In further work we showed that farnesyltransferase inhibitors caused enhanced oxygenation of prostate tumor tissue. This finding is both novel and significant in that hypoxia in tumors is associated with poorer prognosis, metastasis and resistance to both radiation and chemotherapy (25-29). This observation predicts a favorable effect of farnesyltransferase treatment in combinatorial therapies of prostate cancer.

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The Farnesyltransferase Inhibitor L744,832 Reduces Hypoxia in Tumors Expressing Activated H-*ras*[1]

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ABSTRACT

Many tumors contain extensive regions of hypoxia. Because hypoxic cells are markedly more resistant to killing by radiation, repeated attempts have been made to improve the oxygenation of tumors to enhance radiotherapy. We have studied the oxygenation of tumor xenografts in nude mice after treatment with the farnesyltransferase inhibitor L744,832. Hypoxia was assessed by measuring the binding of the hypoxic cell marker pentafluorinated 2-nitroimidazole. We show that xenografts from two tumor cell lines with mutations in H-*ras* had markedly improved oxygenation after farnesyltransferase treatment. In contrast, xenografts from two tumors without ras mutations had equivalent hypoxia regardless of treatment. The effect on tumor oxygenation could be detected at 3 days and remained after 7 days of treatment. These results indicate that treatment with farnesyltransferase inhibitors can alter the oxygenation of certain tumors and suggest that such treatment might be useful in the radiosensitization of these tumors.

INTRODUCTION

Radiation oncologists and radiobiologists have long been interested in tumor oxygenation because of evidence that hypoxic cells may limit radiation treatment outcome. Hypoxic cells survive irradiation to a significantly greater extent than cells in an oxygenated environment. The magnitude of this difference in survival can be on the order of a factor of 2–3-fold *in vitro* (1). Human tumors containing hypoxic cells are more prone to recurrence following radiation therapy. Gatenby *et al.* (2) demonstrated that the short-term clinical response to radiation of well-oxygenated cervical node metastases from head and neck tumors was superior to that of poorly oxygenated lymph nodes. Brizel *et al.* (3) found that disease-free survival in patients with head and neck cancer was better if the tumor was less hypoxic. The average tumor median pO_2 for relapsing patients was 4.1 mm Hg and 17.1 mm Hg in nonrelapsing patients. Additionally patients with hypoxic cervical carcinoma or high-grade soft tissue sarcomas had significantly worse disease-free and overall survival probabilities compared to patients with nonhypoxic tumors after radiotherapy (4–6). Thus, enhanced oxygenation of tumors prior to irradiation could improve tumor cell killing by radiation. The sensitivity of normal tissues would be expected to be unaltered, since normal tissues are usually not hypoxic.

H-*ras* is one of a number of oncogenes which, when activated can contribute to an increased radiation survival in transformed cells (7). Increased clonogenic survival of rodent cells and human cells has been reported after transfection with activated ras (8–11) and inhibition of ras activity in human cells expressing activated ras can radiosensitize these cells (11–14). Since activating mutations of the *ras* family occur in approximately 30% of human tumors, ras is an attractive target for therapeutic intervention. The ras pathway can also be activated by overexpression of ras or through cell surface receptor mutations that lead to deregulated signaling through ras in the absence of *ras* mutation. Thus, inhibiting ras activity may be a therapeutic strategy for a larger number of tumors than those expressing oncogenic ras.

H-ras is prenylated by farnesyltransferase. This is the first and obligate step in ras processing that results in a functional protein (reviewed in Refs. 15–17). Because the addition of a prenyl side chain is a requirement for oncogenic ras transformation, several groups have developed inhibitors specific for one or the other of the two prenyltransferases involved in ras processing. These inhibitors have been isolated as plant metabolites from drug screens and also developed to mimic either the prenyl- group substrate or the tetrapeptide CAAX recognition sequence on ras that is targeted by prenyltransferases. (reviewed in Refs. 18 and 19). By inhibiting ras posttranslational processing, these compounds inhibit ras activity. Farnesyltransferase inhibitors (FTIs)[3] were initially shown to block the growth of *ras*-transformed mouse cells in soft agar and reverse the transformed morphology of v-H-*ras*-transformed fibroblasts, but not to inhibit the growth of *src*- or *raf*-transformed or nontransformed fibroblasts. FTIs have also been shown to inhibit spontaneous tumors in *ras*-transgenic mice (20–22), as well as certain human tumor xenografts in nude mice (23–25).

We have shown that inhibitors of ras prenylation radiosensitize H-*ras*-transformed rat embryo fibroblasts (26) and human tumor cell lines expressing either activated H- or K-ras *in vitro* (13). In our studies, radiosensitization appears to be specific for cells expressing mutated ras and independent of growth inhibition. The radiosensitivity of immortalized cells and primary cells is not diminished by prenyltransferase inhibitor treatment. We have further demonstrated radiosensitization of tumors with mutated H-*ras* by FTIs *in vivo* (27). While part of this effect may be due to alteration in intrinsic radiosensitivity, here we show that treatment of mice with FTIs also leads to reduction of hypoxia in tumors with H-*ras* mutations.

MATERIALS AND METHODS

Tumor Xenograft Generation and FTI Treatment. Pathogen-free Ncr-*nu/nu* mice were obtained from Taconic Farms (Germantown, NY) and housed aseptically. At 5–7 weeks of age, mice were implanted by trocar with 1-mm^3 tumor fragments. Animals were randomly assigned to treatment groups 1–2 weeks after tumor implant when tumors had attained a volume of 100–200 mm^3. FTI treatment consisted of continuous infusion with L744,832 (20, 28) (40 mg/kg/day) or carrier (DMSO:water, 1:1) delivered by Alzet microosmotic pump (Alza Corporation, Palo Alto, CA) for 3 to 7 days. Mice were then injected with 10 mM pentafluorinated 2-nitroimidazole (EF5) in 0.9% saline i.v. (0.01 ml/g body weight), followed by an equal volume i.p. injection 30 min later. Three hours after the first EF5 injection, mice were euthanized

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[3] The abbreviations used are: FTI, farnesyltransferase inhibitor; EF5, pentafluorinated 2-nitroimidazole.

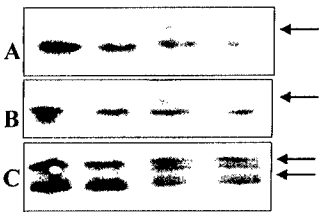

Fig. 1. H-ras farnesylation in tumors is inhibited by FTIs. Mice bearing T24 tumors (*A*), HT-29 tumors (*B*), or 141-1 tumors (*C*) were treated with carrier (*first two lanes*) or with the L744,832 FTI (*third* and *fourth lanes*). L744,832 was administered as a continuous infusion at 40 mg/kg/day by s.c. pump for 3 days. Control mice were treated with continuous infusion of carrier. Tumor xenografts cells were lysed by sonication in 1× reducing Laemmli sample buffer for Western blot analysis. Blots were probed with monoclonal antibody to H-ras. Migration of the unfarnesylated H-ras is indicated by *arrows*. The 141-1 prostate tumor shows two species of ras, both of which undergo a shift in migration after treatment.

and tumors were excised. Samples for Western blot analysis, immunohistochemistry, and flow cytometry were harvested and processed immediately upon sacrifice of the animal. Ras farnesylation was assessed by direct Western blotting of tumor lysates using monoclonal LA069 (Viromed Biosafety, Camden, NJ). Antibody binding was detected using enhanced chemiluminescence (Amersham, Piscataway, NJ).

EF5 Detection of Hypoxia. Frozen tissue sections (10 mm) were cut from the tumor onto poly-L-lysine-coated slides, fixed in 4% paraformaldehyde for 1 h, and then rinsed and blocked for 2 h at room temperature. After removing the block, sections were incubated for 90 min with rat antimouse CD31 (platelet/endothelial cell adhesion molecule 1) monoclonal antibody (PharMingen, San Diego, CA) followed by Cy5-conjugated Affinipure mouse antirat IgG (Jackson ImmunoResearch, West Grove, PA) overnight at 4°C. Slides were then refixed in 4% paraformaldehyde before performing anti-EF5 staining with Cy3-conjugated ELK3–51, a mouse monoclonal antibody to EF5

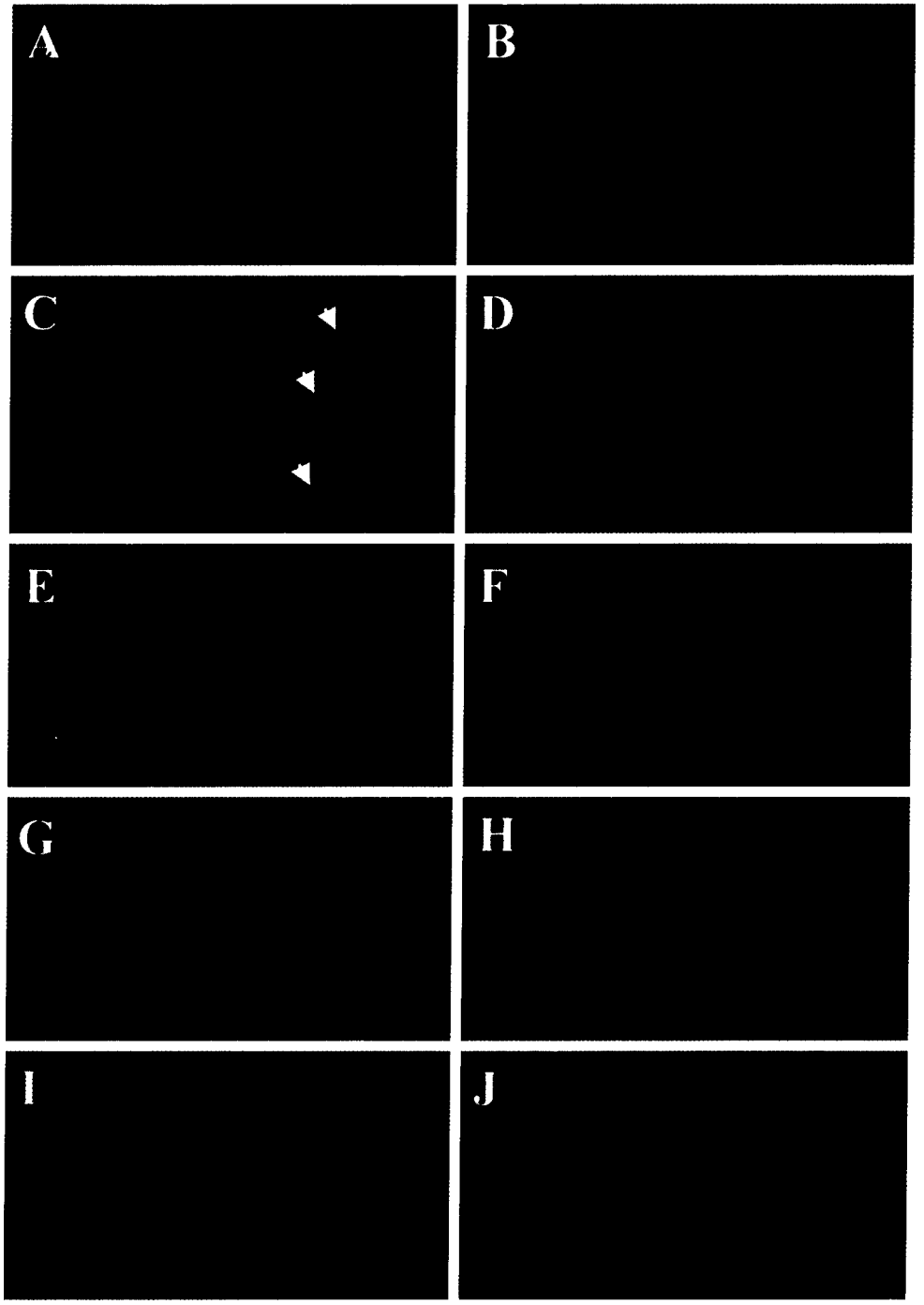

Fig. 2. FTI treatment reduces hypoxia in tumors expressing oncogenic H-ras. Tumor-bearing animals were treated by continuous infusion with L744,832 at 40 mg/kg/day or with carrier (50% DMSO in water). Animals were injected with EF5 and sacrificed after 3 days (*B*) or 7 days (*D*, *F*, *H*, and *J*) of FTI treatment. The control tumors (*A*, *C*, *E*, *G*, and *I*) show extensive binding of EF5 (*red*). Vasculature staining with anti-CD31 (*green*) serves as a counterstain in all tumors. *Arrows* (*C*) denote normal mouse tissue. Exposure times for EF5 photography were increased in tumors with faint EF5 binding. Exposure duration for each frame is as follows: T24, 3-day control: 0.57 s (*A*), 3-day FTI treated: 1.51 s (*B*). T24, 7-day control 0.60 s (*C*), FTI treated: 3.0 s (*D*). 141-1 control: 0.21 s (*E*), FTI treated: 1.78 s (*F*), HT-29 control: 0.79 s (*G*), FTI treated: 0.85 s (*H*), RT4 control: 2.90 s (*I*), FTI treated: 3.54 s (*J*).

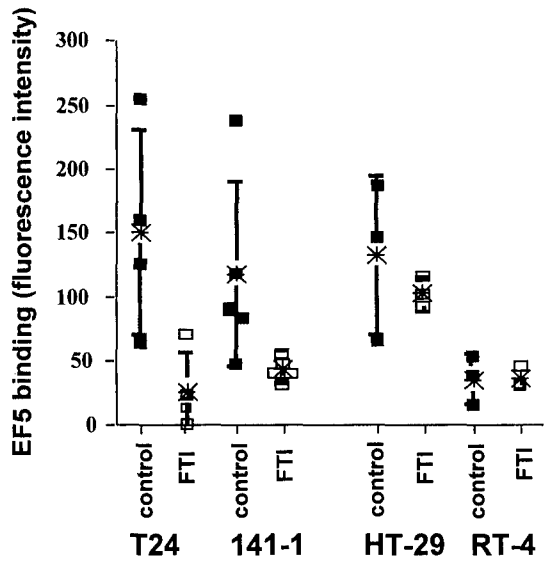

Fig. 3. Tumor oxygenation is increased (EF5 binding is decreased) only in tumors with oncogenic H-*ras*. Mean EF5 staining intensity was quantitated in tumor regions staining with intensities 20 fluorescence channels above background. The mean fluorescence intensities of the positively staining regions in individual tumors is shown. Tumor-bearing mice were treated with 40 mg/kg/day L744,832 for 3 to 7 days (□) and control tumors were treated with carrier alone (■). The mean of values obtained in each group is indicated (*), as is the SD (*bars*). Mann-Whitney *U* analysis demonstrated statistical significance for the difference between FTI-treated and control T24 tumors ($n = 4$ each group; α(D) value of 0.05) and for the difference in FTI-treated and control 141-1 tumors ($n = 5$ each group; α(D) of 0.025).

(29). Stained sections were stored at 4°C in 1% paraformaldehyde until photographed and analyzed.

For flow cytometry analysis, tumors were minced and dissociated for 30 min at 37°C in HBSS containing 166 units/ml collagenase XI, 0.25 mg/ml protease, and 255 units/ml DNase (Sigma, St. Louis, MO). Cells were recovered by straining through an 80-μm mesh, centrifuged at 500 × *g*, and resuspended in culture medium. Single-cell suspensions (10^6 cells) were fixed in 4% paraformaldehyde rinsed, blocked, and stained with Cy5-conjugated ELK3–51. Controls consisted of cells stained in the absence of ELK3–51 (autofluorescence) and cells stained in the presence of saturating amounts of EF5 (background staining). Cells were analyzed on a FACStar flow cytometer and analyzed with CellQuest v3.2 software (Becton Dickinson, San Jose, CA).

Photomicroscopy. Epifluorescence measurements were made using a Nikon LabPhot microscope (Nikon, Melville, NY) with a 100-W high-pressure mercury arc lamp. Filter cubes were optimized for wavelengths of interest (Omega Optical, Brattleboro, VT). Data were captured using a cooled (−25°C) charge-coupled device digital camera (Photometrics Quantix KF1400; Photometrics, Tucson, AZ). Each image field (at ×10 magnification) consisted of 600 × 400 pixels corresponding to 1.05 × 0.7 mm^2. Images were all grayscale TIFF images, containing intensity values from 0 to 255. Images were analyzed using adobe Photoshop software. Variations in the lamp intensity were accounted for by measuring the fluorescence of a reference concentration of Cy3. This reference intensity was used to in each instance to normalize the intensities of images captured from tumor sections at the time of analysis.

RESULTS

Activity of the FTI L744,832 *in Vivo*. Pumps to deliver L744,832 were placed in animals bearing xenografts of tumors derived from T24, HT29, and 141-1. After 7 days of L744,832 (40 mg/kg/day continuous infusion), the animals were sacrificed and the tumors were evaluated for inhibition of H-ras farnesylation. Farnesylated H-ras migrates more rapidly in PAGE than unprocessed H-ras. Fig. 1 shows that treatment of the tumor-bearing animals resulted in accumulation of unprocessed ras in the tumors. This was true regardless of whether the H-*ras* gene contained a mutation and showed that this methodology effectively delivers L744,832 to the tumor.

Oxygenation of Tumors after Treatment with FTI. The extent of hypoxia was evaluated in tumors derived from T24 after 3 or 7 days of L744,832 infusion. Control tumors received the delivery vehicle for the same time. Three hours before sacrifice, the hypoxic cell marker EF5 was administered. Hypoxia was evaluated by fluorescent antibody staining for EF5 bound to tumor cells. Fig. 2, *A* and *C*, show that control tumors after either 3 or 7 days have substantial regions of hypoxia. In contrast, the treated tumors (Fig. 2, *B* and *D*) showed little evidence of hypoxia even with photographic exposure times three to five times longer than controls. The peak fluorescent intensity adjusted for exposure times was calculated by scanning a representative area of 1.05 × 0.7 mm from each of the four tumors in each group. These numbers are plotted in Fig. 3 and indicate that the average peak intensity was reduced 6-fold after treatment with FTI L744,832. FTI L744,832 treatment also reduced hypoxia in tumors derived from 141-1, a murine prostate carcinoma cell line transformed by mutant H-*ras* and v-*myc*. Representative images are shown in Fig. 2, *E* and *F* (the exposure time for the treated tumor is 8.5 × the control exposure). Quantitative image analysis from the tumors is shown in Fig. 3. Reduced hypoxia was also observed in tumors derived from human prostate cells transfected with H-*ras*^{V12}.[4] HT29 (a human colon carcinoma cell line wild type in H-*ras*) resulted in tumors with hypoxia comparable to that seen in T24 and 141-1, yet treatment with L744,832 did not alter this hypoxia (Fig. 2, *G* and *H*; Fig. 3). Tumors from RT4 (a human bladder carcinoma cell line with wild type H-*ras*) were less hypoxic (Fig. 3). The exposures shown in Fig. 2, *I* and *J*, are approximately ×8 the exposure used for HT-29 (Fig. 2, *G* and *H*). This prolonged exposure shows that treatment of mice bearing RT4 tumors with FTI did not alter their hypoxia, which was confirmed with quantitative analysis (Fig. 3).

We confirmed the reduction in tumor hypoxia observed in sections of T24 tumors after FTI treatment by performing flow cytometry on cells isolated from T24 tumors after 7 days of FTI treatment. Cells from L744,832-treated tumors contained only 42% positive cells (Fig. 4*B*) compared to 71% in the untreated tumors (Fig. 4*A*), and the most hypoxic cells (seen at intensities of 10^3-10^4) were drastically reduced.

DISCUSSION

Enhanced of oxygenation of tumors has been a therapeutic goal for many years. In this study, we have shown that FTI treatment of mice bearing xenografts achieved this result. Augmented oxygenation occurred after 3 or 7 days of FTI treatment in mice with tumors derived from cells with activating mutations in H-*ras*. We have previously shown that treatment of tumor cells in cell culture with FTI led to radiosensitization that was dependent upon the presence of a *ras* mutation (13, 26). This sensitization, while significant, was modest and might not have led to detectable radiosensitization *in vivo* after a single dose of radiation, but might have been expected to be revealed only after fractionated treatment. Nonetheless, Cohen-Jonathan *et al.* (27) demonstrated radiation sensitization of H-*ras*-bearing tumors *in vivo* after 3 days of FTI followed by a single dose of radiation. Whereas alteration of the sensitivity of the tumor cells themselves certainly may contribute to the effectiveness of the combined therapy *in vivo*, the results seen here suggest that the unanticipated effect on tumor oxygenation may also contribute to radiosensitization *in vivo*. In support of this, Taxol has also been shown to radiosensitize murine mammary carcinomas coincident with reoxygenation of these tumors (30).

An estimated 30–40 proteins, many unidentified, are farnesylated

[4] Y. Shi, J. Wu, S. M. Evans, C. J. Koch, J. S. Rhim, T. C. Thompson, and E. J. Bernhard, manuscript in preparation.

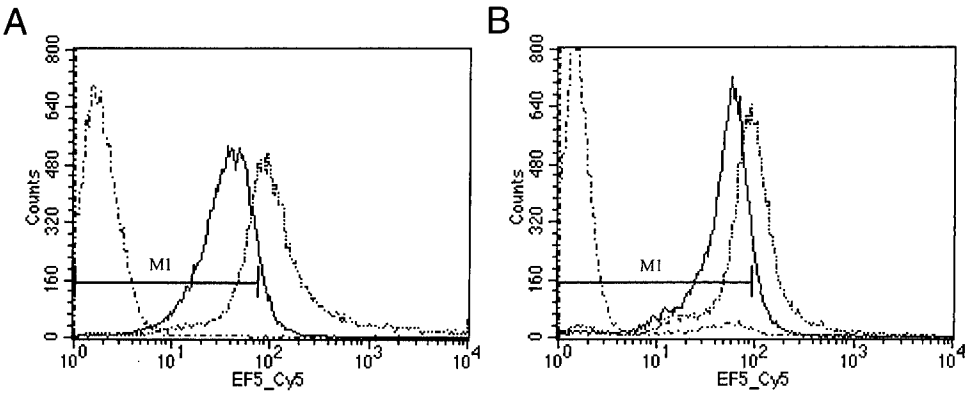

Fig. 4. The severity of hypoxia and the percentage of hypoxic tumor cells are both decreased after FTI treatment of T24 tumor-bearing mice. Mice were treated for 7 days, as described in Fig. 2 legend, with L744,832 or carrier. Tumors were harvested, enzymatically dissociated, and stained for flow cytometry analysis of EF5 binding. Results for T24 control (*A*) and FTI-treated (*B*) tumors show reduced EF5 binding after FTI treatment.

(reviewed in Ref. 31), raising the possibility that proteins other than ras are the target of FTI. Rho B for example has been implicated in the reversal of transformed morphology and the induction of apoptosis by FTI (32–34). The inhibition of tumor cell proliferation also appears to be independent of ras (35). The experiments presented here demonstrate that FTIs can cause increased tumor oxygenation. Although the results are consistent with the idea that H-ras inhibition mediates enhanced tumor oxygenation, they do not prove this point. The results do however suggest that treatment with FTIs may enhance the radiosensitivity of certain tumors by increasing their oxygenation and may thus be useful adjuvants for radiotherapy.

The alterations seen in the tumor microenvironment after FTI treatment might be due to decreased oxygen consumption by the treated tumor cells or altered oxygen delivery. Oncogenic ras has been shown to influence tumor cell metabolism and oxygen consumption (36) and inhibiting ras could decrease oxygen consumption. However, FTI treatment of either T24 or 141-1 cells *in vitro* did not reduce oxygen consumption (data not shown). Inhibiting ras could also influence tumor vasculature (37) or tumor growth leading to altered tumor oxygenation. We are currently investigating the possible mechanisms of FTI-mediated oxygenation of tumors with *ras* mutations.

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37. Gu, W. Z., Tahir, S. K., Wang, Y. C., Zhang, H. C., Cherian, S. P., O'Connor, S., Leal, J. A., Rosenberg, S. H., and Ng, S. C. Effect of novel CAAX peptidomimetic farnesyltransferase inhibitor on angiogenesis in vitro and in vivo. Eur. J. Cancer, *35:* 1394–1401, 1999.

UNIVERSITY OF PENNSYLVANIA - SCHOOL OF MEDICINE

Curriculum Vitae

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Education:

1973-77	B.A. cum laude, University of Utah. (Psychology)
1983-89	Ph.D. University of Virginia (Microbiology and Immunology)

Postgraduate training and Fellowship Appointments:

1989-91	Post-doctoral Fellowship, Tumor Biology Department of Pathology & Lab. Medicine University of Pennsylvania
1992-94	Research Associate, Department Radiation Oncology University of Pennsylvania School of Medicine.
1994-95	Senior Research Investigator, Department Radiation Oncology, University of Pennsylvania School of Medicine.

Military Service: None

Faculty Appointments:

July 2001-	Associate Professor (Research), Department of Radiation Oncology University of Pennsylvania School of Medicine
1995 - 2001	Assistant Professor (Research), Department of Radiation Oncology University of Pennsylvania School of Medicine

Hospital and Administrative Appointments:

1997-1998	Planning Committee for the 1998 Department of Radiation Oncology Retreat
1998	Research Faculty Search Committee for Otorhinolaryngology Department
1995-1996	Department liaison and oversight for $2.5 million Research Lab renovation project

Specialty Certification: None

Licensure: None

Awards, Honors, and Membership in Honorary Societies:

1972	National Merit Scholarship semifinalist
1973	Honors at Entrance, University of Utah
1995	Travel Award, International Congress on Radiation Biology, Wurtzberg, FDR

Membership in Professional and Scientific Societies:

National / International Societies:

1994-present	Member American Association for Cancer Research
1995-present	Member Radiation Research Society
1997-present	Associate Member European Society for Therapeutic Radiology and Oncology
1998-present	Member American Association for the Advancement of Science

Local Societies / Organizations:

1995-present	Member Philadelphia Cancer Research Society
1997-present	University of Pennsylvania Cancer Center
1998-present	University of Pennsylvania Institute for Human Gene Therapy
1998-present	University of Pennsylvania Graduate Group in Cell and Molecular Biology

Editorial Positions: None

Grant review study sections:

March 29, 2000	Member , N.I.H. Radiation Study Section, Special Study Section for review of SBIR grants. Washington, D.C.
July 24, 1999	Member , N.I.H. Radiation Study Section, Special Study Section for review of SBIR grants. Nashville, TN.
June 22, 1999	Temporary Member , N.I.H. Radiation Study Section, Oncological Sciences Initial Review Group

Academic Committees at the University of Pennsylvania:

Special Opportunities Working Group of Faculty 2000

Major Teaching and Clinical Responsibilities for the University of Pennsylvania:

1996-present	Guest Lecturer, Introduction to Radiobiology for Radiology Residents course.
1996-2001	Coordinator, Radiation Oncology Research Seminar Series
1994-96	Co-instructor Immunology 670: Biology of Neoplasia.

Lectures by Invitation:

March 27, 2001	Symposium presentation: Farnesyltransferase inhibitors and the elimination of hypoxic tumor cells. 92nd Annual Meeting AACR, New Orleans LA.
March 5, 2001	The effect of prenyltransferase inhibitors on radiosensitivity, cell signaling and tumor response. 16th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.
June 27, 2000	Farnesyltransferases and Radiosensitization Paul Scherrer Institute, Villigen, Switzerland
June 24-26, 2000	The effects of oncogenic ras on intrinsic radiosensitivity and the tumor microenvironment. International 4th Wolfsburg meeting on Molecular Radiation Biology/Oncology. Ermatingen, Switzerland
June 20, 2000	Farnesyltransferase inhibitors: is the target we aimed for the target we want? Results of preclinical and phase I studies.

	Oncology Day Symposium, Netherlands Cancer Institute Amsterdam, The Netherlands
March 21, 2000	Farnesyltransferase inhibitor effects on tumor radiosensitivity and microenvironment. (Division of Experimental Radiation Oncology Series.) Thomas Jefferson University, Philadelphia, PA.
January 23-30, 2000	*In vivo* tumor response after prenyltransferase inhibitor treatment and PTI-induced changes in the tumor 15th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.
November 16-19,1999	Decreased radiation survival after prenyltransferase inhibition in tumor cells expressing activated ras. Molecular Targets and Cancer Therapeutics Washington, D.C.
October 8-9, 1999	Ras oncogene activation and its contribution to human tumor cell radiation resistance. Predictions of Tumor Response to Therapy: Molecular Markers and the Microenvironment. Montreal, Canada.
January 23-30, 1999	Farnesyltransferase inhibitors as a means of radiosensitizing tumors with ras mutations 14th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.
June 15, 1998	Molecular biology of the cell cycle: Potential for therapeutic applications in radiation oncology. (lecture for: Molecular Mechanisms of Oncogenesis and Radiation Response series.) Thomas Jefferson University, Philadelphia, PA.
January 25-31, 1998	The effects of oncogenes on cellular radiosensitivity 13th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.

December 17-18, 1997	Ras inhibition as a means of radiosensitizing human tumor cells. Visiting Lecturer, Centre de Recherches Claudius Regaud, Toulouse, France
January 28, 1997	13th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.
October 11, 1996	Radiosensitization of tumor cells expressing activated H-ras by farnesyltransferase inhibitor treatment. Anticancer Targets and Strategies for the Twenty-first century. Centre de Recherches Pierre Fabre Castres, France
October 7, 1996	Prenyltransferase inhibitors as tumor cell radiosensitizers Institut Gustave-Roussy, Villejuif, France.
April 16, 1996	Symposium Presentation, Annual Meeting of the Radiation Research Society, Chicago IL
January 10, 1996	Ras activity and radiation sensitivity of tumor cells. 12th CERRO meeting of the European Society for Therapeutic Radiology and Oncology. Les Menuires, France.

Organizing roles in Scientific meetings:

April 29-May 3,2000	Chair, Mini-symposium on Therapy and Clinical Radiology Annual meeting Radiation Research Society Albuquerque,NM
October 25-29, 1998	Moderator, Modifiers of Radiation Response session, American Society for Therapeutic Radiology and Oncology. Phoenix, AZ.
July 11-15, 1998	Co-chair, Mechanisms of Radiation Resistance Symposium, Annual Meeting American Society for Photobiology. Snowbird, UT.

Bibliography:

Research Publications, peer reviewed

Burns, T.F., E.J. Bernhard, W. S. El-Diery. 2001. Tissue specific expression of p53 target genes suggests a key role for KILLER/DR5 in p53-dependent apoptosis *in vivo*. Oncogene (in press)

Liu, A, G. J. Cerniglia, E. J. Bernhard, and G. C. Prendergast. 2001. RhoB is required to mediate DNA damage-induced apoptosis in neoplastically transformed cells. Proc. Natl. Acad. Sci. (USA) (in press).

Gupta, A.K., V.J. Bakanauskas, G.C. Cerniglia, Y.Cheng, E.J. Bernhard, R.J. Muschel,and W.G. McKenna. 2000. The ras radiation resistance pathway. Cancer Res. (in press)

Cohen-Jonathan, E., S.M. Evans, C.J. Koch, R.J. Muschel, W.G. McKenna, J.M. Wu and E. J. Bernhard. 2001. The Farnesyltransferase Inhibitor L744,832 Reduces Hypoxia in Tumors Expressing Activated H-ras. Cancer Res. 61: 2289-2293

Wong, C., A. Lee, L. Shientag, J. Yu, G. Kao, A. B. Al-Mehdi, E.J. Bernhard and R.J. Muschel. 2001. Apoptosis: An early event in metastatic inefficiency. Cancer Res. 61:333-338.

Bernhard, E.J., E.J. Stanbridge, S. Gupta, A.K. Gupta, D. Soto, V.J. Bakanauskas, G.C. Cerniglia, R.J. Muschel, W.G. McKenna. 2000. Direct evidence for the contribution of activated N-*ras* and K-*ras* oncogenes to increased intrinsic radiation resistance in human tumor cell lines. Cancer Res. 60:6597-6600.

Cohen-Jonathan, E., R.J. Muschel, W.G. McKenna, S.M. Evans, G. Cerniglia, R. Mick, D. Kusewitt, S.M. Sebti, A.D. Hamilton, A. Oliff, N. Kohl, J.B. Gibbs and E. J. Bernhard. 2000. Farnesyltransferase inhibitors potentiate the anti-tumor effect of radiation on a human tumor xenograft expressing activated H-ras. Rad. Research 154:125-132.

Gupta, A.K., E.J. Bernhard, V.J. Bakanauskas, J.M. Wu, L.J. Carr, R.J. Muschel,and W.G. McKenna. 2000. Ras-mediated radiation resistance is not linked to MAP kinase activation in two bladder carcinoma cell lines. Rad. Research 154:64-72

Ayene,I.S., E. J. Bernhard, W. G. McKenna., R.J. Muschel, R.E. Krisch, and C.J. Koch. 2000. DNA as an important target in radiation induced apoptosis of myc and myc plus ras transfected rat embryo fibroblasts. Int. J. Radiat. Biol. 76:343-354.

Bernhard, E. J., J. B. Mitchell, D. Deen, M. Cardell, D. I. Rosenthal and J. M. Brown. 2000. Re-evaluating Gadolinium (III) texaphyrin as a radiosensitizing agent. Cancer Res. 60:86-91.

Biaglow, J. E., C. J. Koch, S. W. Tuttle, Y. Manevich, I. Ayene, E. J. Bernhard, W. G. McKenna and A. V. Kachur. 1998. The measurement of bioreductive capacity of tumor cells using methylene blue. Int. J. Radiat. Oncol. Biol. Phys. 42:769-773.

Janss, A. J., C. Levow, M. Haugh, E. J. Bernhard, R.J. Muschel, W. G. McKenna., L. Sutton, and P.C. Phillips 1998. Caffeine and Staurosporine enhance the cytotoxicity of cisplatin and campothecin in human brain tumor cell lines. Exp. Cell Res. 243:29-38.

Bernhard, E. J., W.G. McKenna, A. D. Hamilton, S. M. Sebti, Y. Qian, J. Wu and R.J. Muschel. 1998. Inhibiting ras prenylation increases the radiosensitivity of human tumor cell lines with activating mutations of ras oncogenes. Cancer Res. 58:1754-1761.

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McKenna, W. G., E.J. Bernhard, D. A. Markiewicz, M. S. Rudoltz, A. Maity, R.J. Muschel. 1996. Differential effects of the ras oncogene on apoptosis induction by X-rays and serum withdrawal: Possible role for the radiation induced G2 delay. Oncogene 12:237-245.

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Bernhard, E.J., S. B. Gruber, and R. J. Muschel. 1994. Direct evidence linking MMP-9 (92 kDa gelatinase/collagenase) expression to the metastatic phenotype in transformed rat embryo cells. Proc. Natl. Acad. Sci. U.S.A. 91:4293-4297.

Himelstein, B.P., R.C. Soler, E.J. Bernhard, and R. J. Muschel. 1994. Induction of fibroblast 92 kDa gelatinase/type IV collagenase expression by direct contact with metastatic tumor cells. J. Cell Science. 107:477-486.

Zhou, H., E. J. Bernhard, F. E. Fox, and P. C. Billings. 1993. Induction of metalloprotease activity in human T lymphocytes. Biochim. Biophys. Acta. 1177:174-178.

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Hogan, K. T., C. Clayberger, E. J. Bernhard, S. F. Walk, J. P. Ridge, P. Parham, A. M. Krensky, and V. H. Engelhard. 1989. A Panel of unique HLA-A2 mutant molecules define epitopes recognized by HLA-A2-specific antibodies and cytotoxic T lymphocytes. J. Immunol. 142: 2097-2104.

Le, A-X. T., E. J. Bernhard, M. J. Holterman, S. Strub, P. Parham, E. Lacy, and V. H. Engelhard. 1989. Cytotoxic T cell responses in HLA-A2.1 transgenic mice: Recognition of HLA alloantigens and utilization of HLA-A2.1 as a restriction element. J. Immunol. 142: 1366-1371.

Hogan, K. T., C. Clayberger, E. J. Bernhard, S. F. Walk, J. P. Ridge, P. Parham, A. M. Krensky, and V. H. Engelhard. 1988. Identification by site-directed mutagenesis of amino acid residues contributing to serologic and CTL-defined epitope differences between HLA-A2.1 and HLA-A2.3. J. Immunol. 141: 2519-2525.

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Editorials, Reviews, Chapters:

Bernhard, E.J., Muschel, R.J. Ras, Metastasis and metalloproteinases (MMP-9). 2001. In *Methods in Enzymology.* W.E. Balch, C. J. Der, and A.Hall eds. Academic Press, NY, NY. (in press)

Gupta, A.K., Bakanauskas, V.J., McKenna, W.G., Bernhard, E.J. and Muschel, R.J. 2001. Ras regulation of radioresistance in cell culture. In *Methods in Enzymology.* W.E. Balch, C. J. Der, and A.Hall eds. Academic Press, NY, NY. (in press)

Bernhard, E.J., Muschel, R.J., Cohen-Jonathan, E. , Favre, G., Hamilton, A.D., Sebti, S., and McKenna, W. G.2000. Prenyltransferase inhibitors as radiosensitizers. In *Prenyltransferase Inhibitors in Cancer and Cardiovascular Therapy.* S.M. Sebti and A.D. Hamilton, eds. Humana Press Inc. Totowa, NJ.

Gupta, A.K., Harris, E.E.R., Bernhard, E.J., Muschel, R.J., and McKenna, W. G. 2000. Overview of cell cycle and apoptosis. In *Lung Cancer: Principles and Practice* 2nd Edition. pp 67-81. Pass, Mitchell, Turrisi and Minna eds. Lippincott Williams & Wilkins. Philadelphia,PA.

Bernhard, E.J., McKenna, W. G., and Muschel, R.J. 1999. Radiosensitivity and the cell cycle. The Cancer Journal 5:194-204.

Cohen-Jonathan, E., Bernhard, E.J. and McKenna, W. G. 1999. How does radiation kill cells? Current Opinions in Chemical Biology. 3:77-83.

Muschel, R. J., D. E. Soto, W.G. McKenna and E. J. Bernhard. 1998. Radiosensitization and Apoptosis. Oncogene 17:3359-3363.

Muschel, R. J., W. G. McKenna and E. J. Bernhard. 1997. Cell cycle checkpoints and apoptosis: Potential for improving radiation therapy. In *Vitamins and Hormones* 53: 1-25 Gerald Litwack ed. Academic Press. New York.

Himelstein, B.P., R.C. Soler, E.J. Bernhard, D.W. Dilks, and R. J. Muschel. 1995. Metalloproteinases in tumor progression: The contribution of MMP-9. Invasion and Metastasis. 14:246-258.

Bernhard, E.J., A. Maity, R.J. Muschel and W.G. McKenna. 1995. Effects of ionizing radiation on cell cycle progression. Radiat. and Environ. Biophys. 34:79-83.

Bernhard, E.J., W.G. McKenna, and R.J. Muschel. 1994. Cyclin Expression and G2-phase delay after irradiation. Radiat. Res. Supplement 138:S64-S67.

Engelhard, V. H., E. J. Bernhard, M. J. Holterman, A-X. T. Le, R. Henderson, J. P. Ridge, S. Strub, J. A. Barbosa, and E. Lacy. 1990. Cytotoxic T cell responses against human class I molecules in normal and HLA-A2.1 transgenic mice. In *Transgenic Mice and Mutants in MHC Research*. I. Egerov and C. S. David, eds. Springer-Verlag, Berlin.

Abstracts:

Gupta, A.K., E.J. Bernhard, V.J. Bakanauskas, G.C. Cerniglia, A. Zimmer, R.J. Muschel,and W.G. McKenna. 2000. C-raf-1 protein kinase is not essential for Ras transformation of mouse embryo fibroblasts. (submitted for publication)

Hahn, S. M., E.J. Bernhard, E. Cohen-Jonathan, D.G. Haller, J.P. Stevenson, D. Smith , R.J. Muschel, W.G. McKenna. 2000 *In Vivo* and Clinical Studies of Farnesyltransferase Inhibition and Radiation. (submitted for publication)

Stephen M. Hahn, Eric Bernhard, Krystyna Kiel, Briggs W. Morrison, Mohammed Mohiuddin, Thomas F. Delaney, Deborah Smith, Rebecca Brown, Barnali Pramanik, Paul Deutsch, Ruth Muschel, and Gillies McKenna. Preclinical & phase i trial results of the FTI L-778,123 and radiotherapy. 92nd AACR Annual Meeting, New Orleans, LA. March 24-28, 2001.

Burns, T.F., Bernhard, E.J., and El-Deiry, W.S. Tissue-selective utilization of p53-target gene activation during apoptosis in-vivo. 10th International p53 Meeting. April 2000.

Burns, T.F., Bernhard, E.J., and El-Deiry, W.S. Real-time expression of p53 target genes and caspase activation *in vivo* after apoptotic stimuli. 91st AACR Annual Meeting San Francisco, CA. April 1-5, 2000.

Cohen-Jonathan, E., Evans, S.M., Koch, C., .Muschel, R.J, McKenna, W.G. Gibbs, J.B. and Bernhard, E.J. The farnesyltransferase inhibitor 1744,832 induces reoxygenation in tumors expressing activated H-ras. 91st AACR Annual Meeting San Francisco, CA. April 1-5, 2000.

Bernhard,E.J., Cohen-Jonathan, E., Evans, S.M., Koch, C., Gibbs J.B., Muschel, R.J. and McKenna,W.G. The reoxygenation of tumor xenografts expressing activated H-ras after farnesyl-transferase inhibitor treatment. 47th Annual meeting Radiation Research Society, Albuquerque, NM. April 29th-May 3rd 2000.

Burns, T.F., Bernhard, E.J., and El-Deiry, W.S. The role of p53 and downstream genes in the response to genotoxic stress. 47th Annual meeting Radiation Research Society, Albuquerque, NM. April 29th-May 3rd 2000.

Cohen-Jonathan, E., Muschel, R.J., McKenna, W.G., Hamilton, A.D., Sebti S.M., Gibbs J., Oliff, A., Cerniglia, G., Knight, L., Mick, R. and Bernhard, E.J. Farnesyltransferase mediated radio-sensitization of human tumor xenografts expressing mutant H-ras. Molecular Targets and Cancer Therapeutics Washington, D.C. November 16-19, 1999.

Books: None

Alternative Media None

Patents Sensitization of Cells to Radiation Therapy:

US and European patents pending (applications # 08/839,248 and #209596.0117/2EP)

Australian patent 714560

Invention Disclosures:

Molecular targets for protection from radiation and p53-induced toxicity and apoptosis. (Penn docket N2381)

Modulation of Tumor Cell Hypoxia and Vasculature by Farnesyltransferase Inhibitors (Penn Docket M2148)

Inhibition of Collagenase Expression and Metastasis by Farnesyltransferase Inhibitors (Penn Docket M2181)

PAST GRANT SUPPORT

Eric Jacques Bernhard | Radiation Oncology | 7/10/00

Name of Grant	Period of Award	Grant Category	Role in Grant	%Effort	Funding Source	Current Annual Direct Costs
Radiosensitization of Human Prostate Tumor Cells by Prenyltransferase Inhibitors PC970493	10/1998-3/2001	FG	P.I.	30	DOD	$74,836

CURRENT GRANT SUPPORT

Eric Jacques Bernhard | Radiation Oncology | 7/10/00

Name of Grant	Period of Award	Grant Category	Role in Grant	%Effort	Funding Source	Current Annual Direct Costs
Tumor Radiosensitization Inhibitors RO1 CA73820	12/97-12/2001	**RO1**	P.I.	32	NIH	$133,644
Cell Cycle Targets for Manipulation of Drug and Radiation Sensitivity PO1 CA75138	7/1998-6/2003	**PP**	co-project leader and core director	18	NIH	$767,090
Specialized Center of Research In Hyperbaric Oxygen Therapy Sponsored Research	9/1/00-8/31/05	**FG**	project leader	20	NIH	$144,880
With Merck Support (Prenyltransferase Inhibitors)	10/1998-3/2001	**IG**	co-P.I.	0	Merck & Co. Inc	$107,142

PENDING GRANT SUPPORT

Eric Jacques Bernhard | Radiation Oncology | 7/10/00

Name of Grant	Period of Award	Grant Category	Role in Grant	%Effort	Funding Source	Current Annual Direct Costs
Tumor Oxygenation After Prenyltransferase Inhibitor Treatment 1RO1 CA90905-01	9/1/01-8/31/06	**RO-1**	P.I.	25	NIH	$150,000

OTHER SUPPORT

Eric Jacques Bernhard | Radiation Oncology | 7/10/00

N/A

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 26 Aug 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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